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(54) Title: METHOD OF TREATING RESTENOSIS BY ANTISENSE TARGETING OF CMV

(57) Abstract

A method of inhibiting restenosis in a CMV-infected subject undergoing, or having undergone, angioplasty or atherectomy is described. The subject is treated, preferably at the site of the operation, with an oligomeric molecule effective to specifically hybridize to all or part of a target nucleic acid sequence derived from a human cytomegalovirus (HCMV) gene. The oligomeric molecule is preferably a morpholino oligomer composed of morpholino subunits, each containing a purine or pyrimidine base-pairing moiety, joined by phosphoramidate linkages.

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METHOD OF TREATING RESTENOSIS BY ANTISENSE TARGETING OF CMV

Field of the Invention

The present invention relates to methods and devices for treating or preventing post-angioplasty restenosis by antisense targeting of human cytomegalovirus.

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Background of the Invention

Coronary angioplasty, also known as balloon angioplasty, is a widely used medical procedure for the reopening of arteries clogged by atherosclerotic plaques. Although the initial success rate is high, reocclusion of the vessel, or restenosis, occurs in a large percentage (25-50%) of patients within approximately six months. It is generally accepted that this reocclusion is initiated by injury-induced proliferation of smooth muscle cells (SMC) within the vessel wall. However, the exact cause or causes of such proliferation are not thoroughly understood.

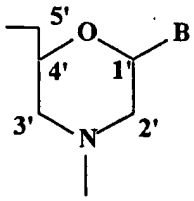
Methods of controlling or inhibiting restenosis have been actively sought. Therapies using various classes of pharmacological agents, including antiplatelet agents, anticoagulants, growth factor antagonists, angiotensin-converting enzyme inhibitors, lipid lowering agents, calcium channel antagonists, and vasodilators, have been explored with limited success (Feldman). Mechanical approaches, such as the deployment of a permanent stent within the treated artery, have shown some success but carry the attendant risks of thrombosis or excessive bleeding.

Thus, there remains a significant need for effective antirestenosis therapy, to reduce post-angioplasty reocclusion and the cost and discomfort associated with repeated angioplasty or surgery.

Summary of the Invention

The present invention includes, in one aspect, a method of inhibiting restenosis in a CMV-infected subject undergoing, or having undergone, angioplasty or atherectomy. According to the method, the subject is treated with a therapeutically effective amount of an oligomeric molecule effective to specifically hybridize to all or part of a selected target nucleic acid sequence derived from a CMV gene. The oligomeric molecule is composed of a sequence of purine and pyrimidine heterocyclic bases, supported by a backbone, which are effective to hydrogen-bond to corresponding, contiguous bases in the target sequence. The backbone is composed of subunit backbone moieties supporting the purine and pyrimidine heterocyclic bases at positions which allow such hydrogen bonding. These backbone moieties are cyclic moieties of 5 to 7 atoms in length. In accordance with the method, administration of the oligomeric molecule is effective to inhibit replication of cytomegalovirus in the subject, and to reduce post-angioplasty restenosis.

A preferred oligomeric molecule is a morpholino oligomer composed of morpholino subunit structures of the form:



where (i) the structures are linked together by uncharged, phosphorous-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and (ii) B is a purine or pyrimidine base-pairing moiety effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide. Preferably, the phosphorus-containing linkages are phosphoramidate linkages.

Preferred CMV target sequences are those included in one of the following coding regions of the CMV genome: the IE1 region, IE2 region, DNA polymerase region, UL36 region, UL37 region, or UL84 region. Preferably, the target nucleic acid sequence is included in a CMV mRNA.

In further preferred embodiments, this sequence includes the promoter region of an mRNA encoding an IE1 or IE2 protein, or it incorporates the 5' untranslated region through the AUG initiator site of a mRNA encoding an IE1 or IE2 protein.

Additional preferred embodiments include the administration of an oligomer as described above, in which the sequence of purine and pyrimidine heterocyclic bases is selected from the following sequences: GCG TTT GCT CTT CTT CTT GCG, GTT TTG CGC GGT TTG TTA CGC, GCG CAC CAT GAC CTG TTT GGG, CGG CTC AGG TCG TCA ATC TTG, GGG TCC TTC ATC TGG GAG AGC, GGT ACT TAC GTC ACT CTT GGC, CCG CGC CCT CTT GTT TGC CGG, CGG CGC AGA TTG CAA GGG CGG, GTG GGC CAT GAT GAT GGA AGG, TGG GGC TTA CCT TGC GAA CA, TCT TCA ACG ACG TGG GGC TT, or GAC GCG TGG CAT GCT TGG TGT (SEQ ID NOS 1-12, respectively). Each of these sequences is complementary to a sequence within one of the CMV coding regions noted above.

The oligomeric molecule, as described, is preferably administered to the subject concurrent with, and at the site of, the angioplasty. In this respect, it may be administered via a perforated or porous catheter balloon, or within a biocompatible polymeric carrier. In the latter case, the carrier may be a hydrogel, e.g. an ethylene oxide/propylene oxide block copolymer. The carrier may also form all or part of an implanted endovascular support device or stent.

In another aspect, the invention provides a drug delivery device for use in inhibiting restenosis following angioplasty in a CMV-infected subject. Such a device includes a catheter balloon or stent, which includes, and is effective to deliver to the site of angioplasty, a therapeutically effective amount of a CMV-targeted oligomeric molecule as described above. The oligomeric molecule is preferably a morpholino oligomer, as described above. The nucleic acid sequences targeted by the

oligomer include those derived from the IE1 or IE2 region, DNA polymerase region, UL36 region, UL37 region, or UL84 region of a CMV gene.

In one embodiment of the device, the catheter balloon is perforated or porous, and the oligomeric molecule is contained within the balloon. Alternatively, the oligomeric molecule is contained in a biocompatible polymeric carrier on the surface of the balloon. The carrier is preferably a hydrogel, *e.g.* an ethylene oxide/propylene oxide block copolymer. When a catheter stent is used, the stent preferably includes, or is composed of, a biocompatible polymeric carrier for the oligomeric molecule.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 shows a repeating subunit segment of a representative antisense morpholino oligomer for binding target mRNA; and

Figure 2 shows catheter-based delivery devices which may be used to deliver antisense compositions to the site of angioplasty in a patient.

Detailed Description of the Invention

I. Antisense Oligomers Targeting CMV Genes

A correlation has recently been described between infection with CMV (cytomegalovirus) and the proliferation of smooth muscle cells that contributes to post-angioplasty restenosis (Speir, Zhou). Human CMV is a herpesvirus with a 230 kbp genome, which has been studied extensively (see, for example, Alford, Chee), and is widespread in the human population.

CMV infection results in a temporally regulated cascade of gene expression, with three major phases defined as immediate early (IE), early, and late. The IE genes play an important regulatory role in expression of the early and late genes. In particular, major proteins expressed by the IE1 and IE2 regions are transcriptional activating factors for other CMV genes. They are believed to transregulate viral and host gene expression as well and thus play an important role in regulating CMV infection.

Accordingly, antisense therapies directed against CMV infection have commonly targeted the IE genes (Anderson, 1992, 1995, 1996; Draper; Bryant), although other regions of the genome, such as the DNA polymerase region, the UL36/37 region, and the UL84 region, have also been targeted (Dal Monte, Smith, Ripalti, Pari). Antisense RNA may be administered directly, or it may be expressed within the cell via transcription of expression vectors prepared from antisense oligonucleotides (Bryant, Dal Monte).

According to the present invention, an antisense oligomeric molecule is administered to an angioplasty patient, preferably at the site of angioplasty, which is specifically hybridizable with a target HCMV DNA or RNA sequence, and preferably a mRNA sequence. In general, the oligomeric molecule is composed of a sequence of purine and pyrimidine heterocyclic bases effective to hydrogen-bond to corresponding, contiguous bases in the target sequence, and a supporting backbone, composed of subunit backbone moieties supporting the purine and pyrimidine heterocyclic bases at positions which allow such hydrogen bonding, where the subunit backbone moieties are cyclic moieties of 5 to 7 atoms in length. The target sequence is preferably within the IE1 or IE2 region of the gene, although other regions may be effectively targeted, as noted above.

Although double-stranded DNA may be targeted by antisense molecules, mRNA transcribed from the relevant region of the gene is more generally targeted. Such mRNA contains, in addition to coding sequences, initiator or promotor sites, intron/exon junction sites, a 3'-untranslated region, and a 5'-untranslated region, which regions may also be targeted. Accordingly, preferred target sequences include all or part of the promoter region of a mRNA encoding an IE1 or IE2 protein, or all of part of the 5'-untranslated region through the AUG initiator site of a mRNA encoding an IE1 or IE2 protein. It has also been found effective (Anderson, 1992) to target a sequence encoding the nuclear localization signal of a protein, which is a region of the protein which facilitates passage through the nuclear membranes of a cell.

Although such an oligomeric molecule is not necessarily 100% complementary to the target sequence, it must be effective to stably and specifically bind to the target sequence such that expression of the sequence to produce, for example, the IE1 or IE2 proteins, is inhibited. The appropriate length of such a molecule to allow stable, effective binding combined with good specificity is about 10 to 40 nucleotide base units, and preferably about 15 to 25 base units.

The effectiveness of a given oligomeric molecule in inhibiting HCMV replication may be determined by screening methods known in the art. For example, to determine effectiveness in inhibiting expression of the targeted sequence, the test molecule is incubated with a cell culture infected with HCMV, and the presence or absence of the encoded protein is determined by standard techniques such as ELISA or Western blotting (see, for example, Pari; Anderson, 1996).

Candidate compounds are also evaluated, according to well known methods, for acute and chronic cellular toxicity, such as the effect on protein and DNA synthesis as measured via incorporation of ^{3}H-leucine and ^{3}H-thymidine, respectively.

It is generally desirable that non-specific binding of the oligomeric molecule to non-target sequences is limited. Although some non-sequence-specific interactions of such oligomers have shown therapeutic effects (*e.g.* Anderson, 1996) such interactions more often produce unwanted side effects. To test for non-specific binding effects, control sequences such as sense or nonsense

sequences, or sequences containing mismatched bases, may be included in screening tests. Excess targeted protein or mRNA may also be added to the cell culture to see if the effect of the antisense oligomer is reversed (Bennett).

Oligomers having the base sequences shown in Table I have been reported effective in inhibiting HCMV replication. The oligomer base sequences and target sequences described herein, though representative, are not considered to be exhaustive. Additional sequences may be prepared by one of skill in the art, having in mind a desired HCMV target sequence, and screened according to methods such as those reported above.

Table 1

Seq ID No.	Target[a]	Sequence	Ref.[b]
1	IE2 nuc sig 2	GCG TTT GCT CTT CTT CTT GCG	1
2	IE2 nuc sig 1	GTT TTG CGC GGT TTG TTA CGC	1
3	IE2 int/exon 2	GCG CAC CAT GAC CTG TTT GGG	1
4	IE2 int/exon 1	CGG CTC AGG TCG TCA ATC TTG	1
5	IE2 AUG	GGG TCC TTC ATC TGG GAG AGC	1
6	IE1 int/exon 1	GGT ACT TAC GTC ACT CTT GGC	1
7	DNA polymerase	CCG CGC CCT CTT GTT TGC CGG	1
8	DNA polymerase	CGG CGC AGA TTG CAA GGG CGG	1
9	DNA polymerase	GTG GGC CAT GAT GAT GGA AGG	1
10	UL36/37 int/exon	TGG GGC TTA CCT TGC GAA CA	2,3
11	UL36/37 int/exon	TCT TCA ACG ACG TGG GGC TT	3
12	UL84 AUG	GAC GCG TGG CAT GCT TGG TGT	3

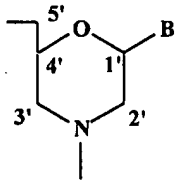
[a] nuc sig = nuclear localization signal; int/exon = intron/exon junction region; AUG = AUG start codon
[b] Ref. 1: Anderson, 1992; Ref. 2: Smith; Ref. 3: Pari

A. Morpholino Oligomers

The antisense binding studies noted above generally employed phosphorothioate-linked oligonucleotide analogs, in which the oxygen atom in each phosphate linkage of an oligonucleotide is replaced by sulfur. These compounds have relatively high nuclease stability, unlike natural oligonucleotides, and good aqueous solubility. However, their correspondingly low lipophilicity, due to the ionic backbone, limits their uptake into cells, which is less efficient than "natural" DNA. The negatively charged backbone also promotes non-sequence-specific interactions of these molecules with cellular proteins (Taylor).

Non-ionic oligonucleotide analogs include phosphotriester- and methylphosphonate-linked DNA (Miller), carbamate-linked DNA (Stirchak), and phosphoramidate-linked DNA (Froehler). Although more readily able to cross cell membranes, these non-ionic nucleic acid analogs bind their target sequences with appreciably lower affinities than do corresponding natural nucleic acids.

A preferred antisense agent should have a high affinity for its target sequence. In this regard, morpholino oligomers, such as illustrated in Fig. 1, afford exceptional target binding affinity, especially for RNA targets. They are also highly resistant to degradation by nucleases (Hudziak). These compounds are composed of morpholino subunit structures of the form:



where (i) the structures are linked together by uncharged, phosphorous-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and (ii) B is a purine or pyrimidine base-pairing moiety effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide. The purine or pyrimidine base-pairing moiety is typically adenine, cytosine, guanine, uracil or thymine. Preparation of such oligomers is described in detail in U.S. Patent No. 5,185,444 (Summerton and Weller, 1993), which is hereby incorporated by reference in its entirety. As shown in this reference, several types of nonionic linkages may be used to construct a morpholino backbone. Fig. 1 illustrates a repeating subunit segment of a morpholino oligomer having a phosphoramidate linkage, which is the preferred linkage.

Although targeting of a messenger RNA sequence is preferred, a double-stranded DNA may be targeted by using a non-ionic probe designed for sequence-specific binding to major-groove sites in duplex DNA. Such probe types are described in U.S. Patent No. 5,166,315 (Summerton and Weller, 1992), which is hereby incorporated by reference.

Thus, morpholino oligomers provide several advantages over more conventional antisense agents. Binding to the target generally gives substantially better target inactivation, due to the greater binding affinity noted above, and because the oligomer/target duplex is not susceptible to duplex unwinding mechanisms in the cell. Further, in therapeutic applications involving cellular uptake of the compound, the uncharged morpholino polymer is more able to enter cells than a charged oligonucleotide or oligonucleotide analog.

B. Preparation of Antisense Oligomers

Antisense oligomers having the various linkages described above may be prepared according to known methods. For example, the widely used phosphorothioate-linked oligonucleotide analogs

may be prepared on commercial DNA synthesizers, available from Applied Biosystems Inc. or Pharmacia, using standard phosphoramidite or beta-cyanoethyl phosphoramidite chemistry. The phosphite linkages are converted to phosphorothioates by oxidizing with 3H-1,2-benzodithiol-3-one-1,1-dioxide in place of the standard iodine reagent (see, *e.g.*, Agrawal, Smith, Iyer).

Morpholino oligomers, the preferred antisense agents, are prepared according to methods described in U.S. Patent No. 5,184,444 (Summerton). Briefly, the morpholino subunits are formed by reacting the corresponding base-protected ribonucleosides with sodium periodate to form a transient 2', 3'-dialdehyde, which is condensed with ammonia to form a morpholino ring having 2' and 3' hydroxyl groups, which are then reduced with sodium cyanoborohydride. The remaining 5'-hydroxyl may be converted to an amine or thiol group, if desired, and any of these functional groups used to form a variety of intersubunit linkages, including the preferred phosphoramidate linkage, as described in the reference.

II. Administration

A. Delivery Devices and Methods

An important aspect of successful anti-restenosis therapy, particularly with regard to antisense agents, is effective delivery of the antisense oligomer to the affected cells. Systemically administered anti-restenosis drugs typically fail to achieve an effective concentration at the site of vessel injury without using unacceptably high concentrations of the drug.

Accordingly, the invention also provides a drug delivery device containing a CMV-targeted antisense oligomer, as described above, effective to deliver the oligomer to the site of angioplasty in a patient. The oligomer is preferably a morpholino oligomer, and is preferably contained in a pharmaceutically acceptable carrier. In a preferred method of the invention, such a device is used to deliver the therapeutic oligomer concurrent with the angioplasty procedure.

Suitable devices, as shown in Fig. 2, include perforated (Fig. 2A) or porous (Fig. 2B) catheter balloons containing the oligomer formulation (see, for example, Heuser, Feldman, and references cited therein). A porous balloon, or a perforated balloon covered with a porous membrane, is preferred in that it reduces "jetting", or administration of the drug at excessive pressures to overly localized points within the vessel wall.

Certain balloon designs allow different pressures to be used for the angioplasty procedure and for drug delivery, to prevent further vessel injury from excessive pressure during drug delivery. These include balloons with separately pressurizable channels containing the oligomer, such as shown in Fig. 2C, and stents (metal or polymeric endovascular support structures) surrounded by a porous drug-containing balloon, such as shown in Fig. 2D.

As noted above, the antisense oligomer is preferably administered during angioplasty. As reported by Farrell, uptake of an oligonucleotide by cells can be significantly increased when the

compound is administered to balloon-injured arteries, relative to uptake by normal arteries. For prolonged delivery, subsequent to the angioplasty procedure, increased uptake may be effected by increased pressure on the blood vessel, as a further manifestation of this effect. A stent, for example, can maintain pressure against the blood vessel wall and deliver the drug via a surrounding balloon (Fig. 2D) or a drug-eluting polymer, as described below. However, such a benefit must be balanced against the possible harmful effects of additional physical trauma to the vessel.

The drug delivery device may also incorporate a biocompatible polymeric carrier containing the oligomer. Preferred carriers include Pluronic™ hydrogels, which are block copolymers of polyethylene oxide and polypropylene oxide. Over a fairly wide range of compositions, such copolymers are liquid at room temperature and solidify to gels at body temperature. This property allows additives to be easily mixed into the carriers at room temperature; upon delivery to a region within the body, the polymeric carrier solidifies, and the additive diffuses out of the carrier at the desired site of administration. Such a carrier may be coated on a balloon, as illustrated in Fig. 2E, or on a stent, which is delivered via catheter to the angioplasty site. This approach permits prolonged delivery of the therapeutic oligomer to the site, where the rate of delivery is dependent on the composition of the carrier, in this case, the ethylene oxide-polyethylene oxide ratio. Other carriers may also be used; for example, antisense oligonucleotide analogs contained in an ethylene/vinyl acetate copolymer (EVAc, Dupont Co.) were released more slowly than those contained in a Pluronic™ gel (Pluronic F-127, BASF Wyandotte Corp) (Edelman).

Alternatively, a stent may be formed of, or be coated with, a drug-eluting polymer. Suitable biocompatible materials include EVAc, polyvinyl acetate, and ethylene vinyl alcohol. Such a stent may be permanent or removable; biodegradable stents have also been described (see, for example, Tanguay). Suitable biodegradable materials include polyanhydrides, polylactic and polyglycolic acids, and naturally occurring polymers such as collagen and dextran.

B. Formulations and Dosages

The antisense oligomer compositions typically include a pharmaceutical carrier or excipient, which may be a conventional liquid carrier, as described below, or a polymeric carrier as described above. The compositions may additionally include other medicinal agents, adjuvants, and the like. Oligonucleotide/polymeric carrier formulations may be prepared as described in, for example, Rosenberg.

Liquid compositions can be prepared by dissolving or dispersing the compounds (about 0.1% to about 20%), and optional pharmaceutical adjuvants, in a carrier, such as, for example, aqueous saline, aqueous dextrose, glycerol, or ethanol, to form a solution or suspension. The composition may also formulated as a suspension in a lipid or phospholipid, in a liposomal suspension, or in an aqueous emulsion. Such formulations may be employed, for example, in devices such as shown in

Figs. 3A-3D. Delivery of oligonucleotides in liposomes coated with inactivated hemagglutinating virus is also reported to enhance uptake into cells (Morishita).

For systemic delivery, the composition may be prepared as a solution, suspension, or emulsion, being supplied either in liquid form or a dried form suitable for hydration in water or normal saline. Methods for preparing such dosage forms are known or will be apparent to those skilled in the art; for example, see Remington's Pharmaceutical Sciences (18th Ed., Mack Publishing Co., 1995). Although the composition may be administered to a subject by various systemic routes, including oral or parenteral, direct administration to the site of angioplasty, as described in Section A above, is preferred.

Generally, dosages will be determined according to the size of the subject, the route of administration, and the extent of CMV infection, according to standard pharmaceutical practices. The preferred level of drug is that effective to inhibit CMV replication, and reduce or prevent restenosis, without unacceptable side effects. For an adult human, a recommended dosage is in the range of 1-25 μmol of antisense oligomer, and preferably 2-15 μmol. Optimum dosages for a given route can be determined by routine experimentation according to methods known in the art. For example, for delivery to the site of vessel injury, *in vivo* models such as described in Edelman and Rosenberg may be used.

When the oligomer is incorporated into a drug delivery device, as described above, the device is effective to deliver a dosage of drug to the patient, at the site of angioplasty, which is effective to inhibit CMV replication, as noted above, and prevent restenosis. For delivery to the site of angioplasty, the surface area of tissue to be treated is also considered. An effective dose is typically in the range of 30 to 3000 μg oligomer per cm^2 of vessel wall, and more preferably about 300 to 1500 $\mu g/cm^2$. The patient may also be given the composition on a periodic basis after angioplasty, at a dosage level sufficient to further inhibit CMV infection and restenosis.

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Antivirals, Inc.

(ii) TITLE OF THE INVENTION: Method of Treating Restenosis by Antisense Targeting of CMV

(iii) NUMBER OF SEQUENCES: 12

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(v) COMPUTER READABLE FORM:
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(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Not yet assigned
(B) FILING DATE: 16-APR-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/043,274
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGTTTGCTC TTCTTCTTGC G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTTTGCGCG GTTTGTTACG C 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCGCACCATG ACCTGTTTGG G 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGCTCAGGT CGTCAATCTT G 21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGGTCCTTCA TCTGGGAGAG C 21

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTACTTACG TCACTCTTGG C 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCGCGCCCTC TTGTTTGCCG G 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGCGCAGAT TGCAAGGGCG G 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGGGCCATG ATGATGGAAG G 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGGGGCTTAC CTTGCGAACA 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCTTCAACGA CGTGGGGCTT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GACGCGTGGC ATGCTTGGTG T 21

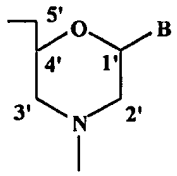
IT IS CLAIMED:

1. A method of inhibiting restenosis in a CMV-infected subject undergoing, or having undergone, angioplasty or atherectomy, comprising administering to such a subject a therapeutically effective amount of an oligomeric molecule effective to specifically hybridize to all or part of a selected target nucleic acid sequence derived from a CMV gene,

wherein said oligomeric molecule is composed of a sequence of purine and pyrimidine heterocyclic bases effective to hydrogen-bond to corresponding, contiguous bases in the target sequence, and a backbone, composed of subunit backbone moieties supporting the purine and pyrimidine heterocyclic bases at positions which allow such hydrogen bonding, where the subunit backbone moieties are cyclic moieties of 5 to 7 atoms in length,

whereby said administration is effective to inhibit CMV replication and restenosis in said subject.

2. The method of claim 1, wherein said oligomeric molecule is a morpholino oligomer composed of morpholino subunit structures of the form:



where (i) the structures are linked together by uncharged, phosphorous-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and (ii) B is a purine or pyrimidine base-pairing moiety effective to bind by base-specific hydrogen bonding to a base in a polynucleotide.

3. The method of claim 2, wherein said phosphorus-containing linkages are phosphoramidate linkages.

4. The method of claim 1, wherein said selected target sequence is included in the IE1 or IE2 region, DNA polymerase region, UL36 region, UL37 region, or UL84 region of a CMV gene or CMV mRNA.

5. The method of claim 4, wherein the target nucleic acid sequence is included in a CMV mRNA.

6. The method of claim 5, wherein said target sequence includes all or part of the promoter region of a mRNA encoding an IE1 or IE2 protein.

7. The method of claim 5, wherein said target sequence incorporates all or part of the 5' untranslated region through the AUG initiator site of a mRNA encoding an IE1 or IE2 protein.

8. The method of claim 5, wherein the sequence of purine and pyrimidine heterocyclic bases in said oligomeric molecule is selected from the group consisting of:
GCG TTT GCT CTT CTT CTT GCG (SEQ ID NO: 1)
GTT TTG CGC GGT TTG TTA CGC (SEQ ID NO: 2),
GCG CAC CAT GAC CTG TTT GGG (SEQ ID NO: 3),
CGG CTC AGG TCG TCA ATC TTG (SEQ ID NO: 4),
GGG TCC TTC ATC TGG GAG AGC (SEQ ID NO: 5),
GGT ACT TAC GTC ACT CTT GGC (SEQ ID NO: 6),
CCG CGC CCT CTT GTT TGC CGG (SEQ ID NO: 7),
CGG CGC AGA TTG CAA GGG CGG (SEQ ID NO: 8),
GTG GGC CAT GAT GAT GGA AGG (SEQ ID NO: 9),
TGG GGC TTA CCT TGC GAA CA (SEQ ID NO: 10),
TCT TCA ACG ACG TGG GGC TT (SEQ ID NO: 11), and
GAC GCG TGG CAT GCT TGG TGT (SEQ ID NO: 12).

9. The method of claim 1, wherein said oligomeric molecule is administered concurrent with and at the site of said angioplasty.

10. The method of claim 9, wherein said oligomeric molecule is administered via a perforated or porous catheter balloon.

11. The method of claim 9, wherein said oligomeric molecule is contained within a biocompatible polymeric carrier.

12. The method of claim 11, wherein said carrier is a hydrogel comprising an ethylene oxide/propylene oxide block copolymer.

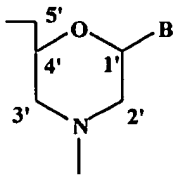
13. The method of claim 11, wherein said polymeric carrier forms all or part of an endovascular support device or stent.

14. A drug delivery device for use in inhibiting restenosis following angioplasty in a CMV-infected subject, said device comprising a catheter balloon or stent containing, and effective to deliver to the site of said angioplasty, a therapeutically effective amount of an oligomeric molecule effective to specifically hybridize to all or part of a target nucleic acid sequence derived from a

CMV gene,

wherein said oligomeric molecule is composed of a sequence of purine and pyrimidine heterocyclic bases effective to hydrogen-bond to corresponding, contiguous bases in the target sequence, and a backbone, composed of subunit backbone moieties supporting the purine and pyrimidine heterocyclic bases at positions which allow such hydrogen bonding, where the subunit backbone moieties are cyclic moieties of 5 to 7 atoms in length.

15. The device of claim 14, wherein said oligomeric molecule is a morpholino oligomer composed of morpholino subunit structures of the form:



where (i) the structures are linked together by uncharged, phosphorous-containing linkages, one to three atoms long, joining the morpholino nitrogen of one subunit to the 5' exocyclic carbon of an adjacent subunit, and (ii) B is a purine or pyrimidine base-pairing moiety effective to bind by base-specific hydrogen bonding to a base in a polynucleotide.

16. The device of claim 14, wherein said selected target sequence is included in the IE1 or IE2 region, DNA polymerase region, UL36 region, UL37 region, or UL84 region of a CMV gene or CMV mRNA.

17. The device of claim 14, wherein said balloon is perforated or porous, and said oligomeric molecule is contained within said balloon.

18. The device of claim 14, wherein said oligomeric molecule is contained in a biocompatible polymeric carrier on the surface of said balloon.

19. The device of claim 18, wherein said carrier is a hydrogel comprising an ethylene oxide/propylene oxide block copolymer.

20. The device of claim 14, wherein said stent comprises a biocompatible polymeric carrier for said oligomeric molecule.

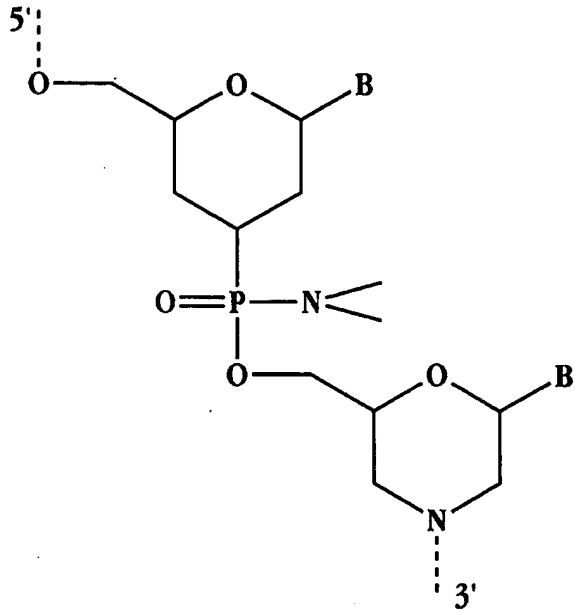


Fig. 1

Fig. 2A

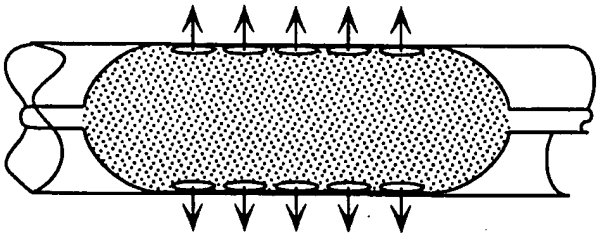


Fig. 2B

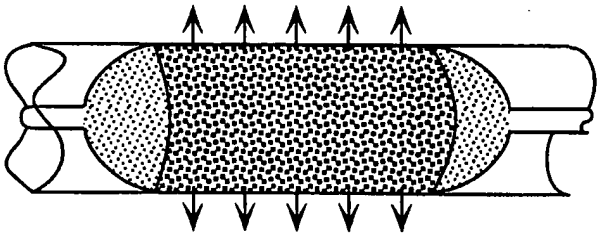


Fig. 2C

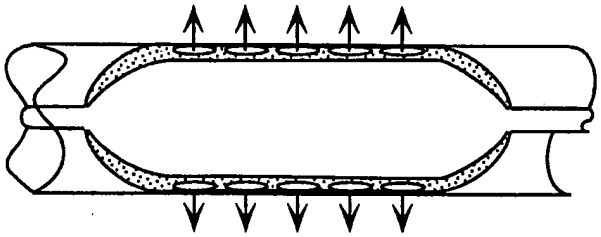


Fig. 2D

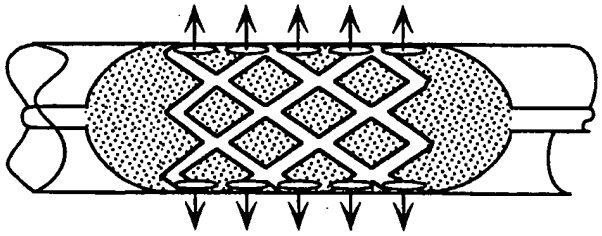
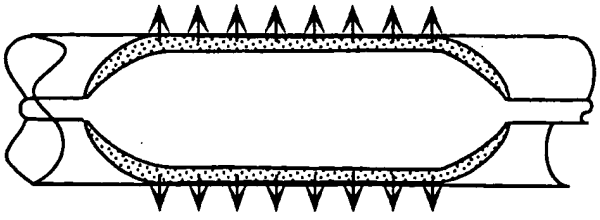


Fig. 2E



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07866

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 A61L29/00 A61M25/10 C07H21/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 22098 A (WELLCOME FOUND ;MURRAY ALISON BRIGID (GB); CHULAY JEFFREY DAVID (U) 25 July 1996 see the whole document ---	1-13
Y	WO 92 03456 A (ISIS PHARMACEUTICALS INC) 5 March 1992 cited in the application see table 2 see SEQ IDs 5,7,11-12,18-22 see examples --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 September 1998	14/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07866

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 01286 A (MASSACHUSETTS INST TECHNOLOGY) 21 January 1993 cited in the application see page 3, line 24 - line 29 see page 6 - page 7 see page 14, paragraph 2 - page 15 see examples 2-6 see claims	9-20
Y	PARI, G. ET AL.: "Potent antiviral activity of an antisense oligonucleotide complementary to the intron-exon boundary of human cytomegalovirus genes UL36 and UL37." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 39, May 1995, pages 1157-1161, XP002076128 cited in the application see table 1	8
A	WO 91 09033 A (ANTIVIRALS INC) 27 June 1991 cited in the application see abstract see page 32, line 11 - page 34 see claims	2,3,15
A	ZHOU, Y. ET AL.: "Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy" THE NEW ENGLAND JOURNAL OF MEDICINE, vol. 335, 29 August 1996, pages 624-630, XP002076129 cited in the application	

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/07866

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1 - 13
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 98/07866

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-18 (totally) and claims 9-13 (partially)
   A method for inhibiting restenosis with antisense oligonucleotides targeted against nucleic acids derived from CMV.

2. Claims: 14-20 (totally) and 9-13 (partially)
   A drug delivery device.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/07866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9622098 A	25-07-1996	NONE	
WO 9203456 A	05-03-1992	AT 154947 T	15-07-1997
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